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Repetitive-Selection Synthesis of Surface Bound Metal Binding Proteins

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**13. ABSTRACT** *(Maximum 200 words)*

A silica surface is reacted with a tridentate siloxane to introduce appropriately spaced tris-aminoalkyl(aryl) groups as a template for protein assembly. The modified surface was reacted with simple aldehyde-modified amino acid derivatives. Only bipyridine-containing aldehyde was reacted with the surface in the presence of Fe(II) ion whereas no selective binding was observed in the absence of metal ions. The selection experiments with these simple aldehyde-modified amino acid derivatives demonstrate the feasibility of the repetitive-selection cycle to assemble a specific metal binding site on silica surface. A three-helix bundle protein was synthesized in solution to test structural stability. The resulting protein was found to be highly helical as expected. The protein, however, appears to be a molten globule instead of a native-like folded state.

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**FINAL TECHNICAL REPORT**

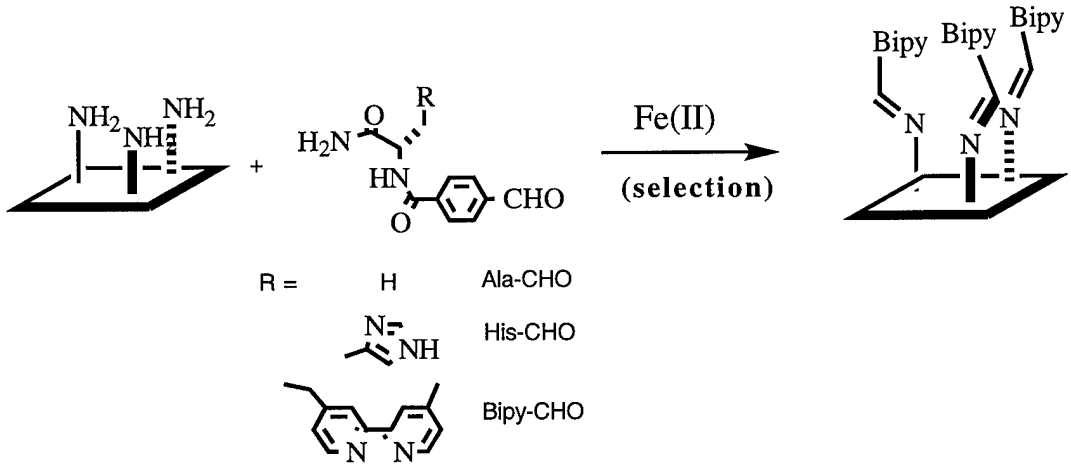
Tomikazu Sasaki, University of Washington

The objectives of the project were 1) to develop synthetic and analytical methods for the assembly of artificial proteins on a solid surface. 2) to synthesize and characterize 3-helix bundle proteins with a metal binding site from a library of peptide segments. 3) to develop a biomimetic repetitive-selection method for the assembly of artificial proteins with desired structural and functional characteristics.

We have synthesized two organic siloxane templates and one inorganic siloxane template for surface modification. p-Methoxydimethylsilylaniline(MODSA) and 3-aminopropyltri-ethoxysilane(APTES) were reacted with TRIPOD, a structurally rigid trialdehyde, to prepare siloxane templates. Molecular imprinting of porous silica gel (Fractosil 500) was carried out with the siloxane-TRIPODs. We discovered that the APTES-TRIPOD partially decomposes during the imprinting reaction to produce p-hydroxybenzaldehyde. The degradation appears to be caused by the partial hydrolysis of the Schiff's base and subsequent attack of the ether linkage by APTES. The new MODSA-TRIPOD was found to be better for molecular imprinting because of its superior stability. After the imprinting, TRIPOD was successfully removed from the surface by mild hydrolysis to leave three amino groups at the imprinting site. The surface-bound dimethylsilylaniline group was confirmed by HF treatment of the modified silica followed by GC-MS analysis.[1] In order to increase sensitivities during surface characterization and analysis, we have also developed a inorganic siloxane template for molecular imprinting. The template was synthesized by reacting 4-formyl-4'-methylbipyridine (FMP) with $RuCl_3$ in ethanol. The resulting Ru(II) complex was treated with excess aminopropyltriethylsiloxane (APTES) to form a Shiff's base. Molecular imprinting of porous silica gel was carried out with the Ru(II) template. After the imprinting, Ru(II) template was removed by a mild acid treatment. The ratio of recovered Ru(II) template and amino groups on the silica surface was found to be 1 : 3 at low substitution level, in accord with the formation of monolayer on the surface. At higher substitution levels, however, more Ru(II) template than expected from the content of surface amino groups was recovered, indicating the formation of multiple layer. We found that the average distance between imprinted site was ca.40Å at an optimum substitution level.[2]

We have completed initial experiments to develop the selection step. $Ru(II)(FMP)_3$ and $Ru(II)(FMP)(bipy)_2$ complexes were synthesized and reacted with the imprinted silica to test the thermodynamic stability of their Shiff bases. In the Schiff's base of $Ru(II)(FMP)_3$, Ru(II) provides crosslinkings between bipyridine aldehydes while $Ru(II)(FMP)(bipy)_2$ should form a simple monodentate Shiff's

base. The relative stability of Shiff's bases of $Ru(II)(FMP)_3$ and $Ru(II)(FMP)(bipy)_2$ complexes was determined by competition. The selectivity factor was found to be ca. 5. To extend the scope of the repetitive-selection approach, several aldehyde-modified amino acid derivatives (Ala-CHO, His-CHO, and Asp-CHO) have been synthesized for the selection experiments. Also, aldehyde-modified bipyridine (bipy-CHO) was also synthesized as a Fe(II)-specific recognition element. The formyl group of p-formyl benzoic acid was protected as a cyclic acetal to avoid the Schiff's base formation during the coupling reaction. The DCC-mediated coupling reaction proceeded in a reasonable yield, and the final deprotection of cyclic acetal with an acid afforded the desired aldehyde-modified recognition elements.




A mixture of Ala-CHO, His-CHO and bipy-CHO was reacted with the imprinted silica in methanol with and without Fe(II). After 20 hrs, supernatant was analyzed by HPLC, showing a selective adsorption of bipy-CHO in the presence of Fe(II). Silica was then isolated, washed with methanol, and treated with an aqueous acid to release any adsorbed aldehydes. HPLC analysis of the released aldehydes showed only bipy-CHO, and no His-CHO and Ala-CHO were detected above noise level. Bipyridine is known to form a very stable Fe(II) complex, thus consistent with the observed selective adsorption of bipy-CHO. On the other hand, no selective binding of aldehydes was observed in the absence of Fe(II). The above selection experiments with simple aldehyde-modified amino acid derivatives demonstrate the feasibility of the repetitive-selection cycle to assemble a specific metal binding site on silica surface.[3] The results suggest that similar selection experiments can be carried out with organic substrates in an aqueous methanol solution. A 15-residue peptide, a building block for 3-helix bundle proteins discussed below, was synthesized using Fmoc-chemistry. p-Formylbenzoic acid (FBA) was coupled to the N-terminus of the 15-residue peptide. The aldehyde-modified peptide and FBA-modified alanine were successfully coupled to the silica

surface which was randomly modified with aminopropyl-triethoxysiloxane. The modified silica gel was characterized by amino acid analysis and $^{13}C$-CPMAS-NMR. We have attempted template-assisted modifications of quartz plates with the Ru(II) template. The substitution level was, however, difficult to control with apparatus currently available in our lab.

We also synthesized a three-helix bundle protein in solution to examine the structural stability during the repetitive-selection cycles. Three 15-residue peptides were attached covalently to a template that used for molecular imprinting. The peptide was designed to form an amphiphilic $\alpha$-helix. The resulting protein was found to be highly helical as expected. The protein, however, appears to be a molten globule instead of a native-like folded state.[4] Incorporation of a metal binding site is expected to stabilize the tertiary structure of the artificial protein.

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